Frequency of bovine lymphocyte antigen DRB3.2 alleles in Sarabi cows

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Abstract
*The second exon of the bovine Major Histocompatibility Complex (MHC) class II DRB3 gene was amplified by polymerase chain reaction (PCR) from 50 DNA samples of Iranian Sarabi cattle. Bovine DNA was isolated from aliquots of whole blood. A two-step polymerase chain reaction followed by digestion with restriction endonucleases RsaI, BstyI, and HaeIII was conducted on DNA from samples. Fifteen Bovine Lymphocyte Antigen (BoLA)-DRB3 alleles were assigned, including some that were only recently described for zebu cattle. Allelic frequencies ranged from 0.02 to 0.23. The most frequent alleles were *52 (frequency = 0.23), *11 (0.18) and *23 (0.15). Results of this study demonstrate that the BoLA-DRB3.2 locus is highly polymorphic in Sarabi cattles.*
Keywords: *BoLA-DRB3.2, polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), Iranian Sarabi cows.*

INTRODUCTION

Molecular techniques have been developed that have resulted in identification of new genetic markers for the characterization of genes responsible for production traits and host immunity (Lewin *et al*., 1989). The major histocompatibility complex (MHC) of cattle is known as Bovine Lymphocyte Antigen (BoLA) and located on chromosome 23 (Lewin *et al*., 1996). The BoLA class II genes encode highly polymorphic transmembrane glycoproteins that present antigenic peptides to helper T cells and thus trigger a humoral immune response. The BoLA-DR region consists of one DRA locus and at least three DRB loci, with exon 2 of the DRB3 gene being highly polymorphic (Maillard *et al*., 1999). Lewin *et al*. (1996) identified 35 DRB3 alleles in exon 2 with a technique described by Van Eijk *et al*. (1992) involving polymerase chain reaction (PCR) and endonuclease restriction fragment length polymorphism (RFLP).

The *BoLA-DRB3.2* locus is highly polymorphic; more than 30 different alleles have been reported. Gelhaus *et al*., (1995) identified fourteen additional novel *BoLA-DRB3.2* alleles. Van Eijk *et al.* (1992) reported thirty different alleles based on evaluation of 168 animals representing 10 cattle breeds including Jerseys. Dietz *et al*. (1997a) indicated that 22 *BoLA-DRB3.2* alleles were detected in Holstein cows from one research herd. *BoLA-DRB3.2*2, *4, *5, *14, *17, *18, *19, *29,* and **30* were detected in the study by Van Eijk *et al*. (1992) but were not observed in Holstein cows from the study by Dietz *et al*. (1997b). In a larger study involving *BoLA-DRB3.2* genotyping of 1100 Holstein cows from 93 commercial dairy farms in the United States, 24 previously described alleles and five new alleles were found. The six most frequently detected alleles (*BoLA-DRB3.2 *8, *11, *16, *22, *23,* and **24*) accounted for 70.3% of the alleles in the population. Sharif *et al*., (1998) and Nassiry *et al*. (2004) reported similar *BoLA-DRB3.2* allele frequencies.

The *BoLA-DRB3.2* allele potentially affects many traits related to immunity, Somatic Cell Count (SCC), and mastitis incidence. Associations have been made with some infectious diseases of cattle and *BoLA*

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genes. Kelm *et al.,* (1997) and Schmutz *et al*. (1992) indicated that one *BoLA-DRB* gene pattern in a study of 106 Holstein cows was associated with resistance to *Staphylococcus aureus* mastitis. Dietz *et al*. (1997b) reported that *BoLA-DRB3.2**8, *16, *22, and *28 alleles were associated with elevated SCC and cows with *BoLADRB3.2*16* and *24 were more susceptible to intramammary infection caused by major mastitis pathogens. Furthermore, cows with *BoLA-DRB3.2*11, *12,* and *23 alleles were more resistant to clinical mastitis and to intramammary infection caused by major mastitis pathogens.

Most of the previous studies of the *BoLA-DRB3.2* gene have involved Holstein cows or a combination of various breeds. Few studies have analyzed exclusively the *BoLA-DRB3.2* gene of indigenous domestic breeds. The Sarabi breed of cattle represents an excellent source of biological information for studies on genetic characterization, as it results form a long process of natural selection. This breed has been kept basically with no selection for productive traits, and it should therefore maintain the genetic variability which has allowed it to adapt and survive in the adverse condition of the Sarab region (East Azarbayejan of Iran). As there are no studies of *BoLA-DRB3* genotyping in the Sarabi breed, the purpose of the present study was to determine the *BoLA-DRB3.2* allele pattern in a herd of Sarabi cattle.

MATERIAL AND METHODS

DNA Isolation: 50 Sarabi cattle from the Station of Sarabi breeding (Sarab) were used in this study. Approximately 15 ml of blood was collected on Ethylendiamine tetraacetate (EDTA) from each animal via the jugular vein, and aliquots of whole blood were stored at –20°C. DNA was isolated from whole blood by Boom method (Boom *et al*., 1989).

***BoLA-DRB3.2* Gene Amplification:** DNA amplification of the *BoLA-RB3.2* gene was achieved by a two-step PCR (Van Eijk *et al*., 1992). The oligonucleotide Primers HL030 (5´-ATCCTCTCTCTGCAGCACATTTCC-3´) and HL031 (5´-TTTAATTCGCGCTCACCTCGCCGCT-3´) were used in the first amplification round. Amplification reactions were carried out with 100 ng of DNA (5 µl) in a 25-µl total volume containing 1 PCR buffer, 10 mM dNTP mix, 50 mM MgCl2, 0.5 µm of each primer and 1 unit of Taq DNA polymerase. The thermal cycling profile for the first round of amplification was an initial denaturation step of 3 min at 94°C followed by 10 cycles of 25 s at 94°C, 30 s at 60°C, 30 s at 72°C and a final extension step of 5 min at 72°C. After the first round, a hemi-nested second PCR reaction was carried out with 3 µl of first-round product into one new tubes containing the same volume and concentration as described above except with primers HL030 and HL032 (5´- TCGCCGCTGCACAGTGAAACTCTC-3´). Primer HL032 is internal to the sequence of the amplified product of the first round PCR and has eight bases that overlap with primer HL031 (underlined in the text above). The thermal cycling profile for the second round was 25 cycles of 40 s at 94°C and 30 s at 65°C, followed by a final extension step of 5 min at 72°C (5). Electrophoresis was carried out on 2% agarose gel with 5 µl of PCR product (Fig. 1) (Van Fick *et al*., 1992).

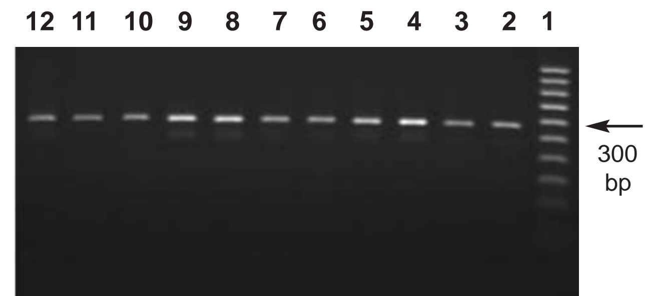

Figure 1. Hemi- nested PCR products. Lane 1 is 100 bp molecular marker. The other lanes are PCR-products of BoLA-DRB3.2 with 284 bp size.

Restriction Endonuclease Digestion: The PCR-amplified DNA fragments from the second PCR reaction were digested with restriction endonuclease *RsaI*, *BstYI*, and *HaeIII*. For the restriction endonuclease digestion reaction, 10 µl of the second PCR reaction product was used for each digestion (Van Fick *et al*., 1992). Samples were digested with *RsaI* or *HaeIII* (10 units) for 3 h at 37°C in a total volume of 20 µl. Samples were digested with *BstYI* (10 units) for 5 h at 50°C in a total volume of 20 µl.

Acrylamide Gel Electrophoresis: Restriction enzyme digested samples were electrophoresed in 8% polyacrylamide with TBE buffer (0.9 M Tris base, 0.09 M boric acid, 2.5 mM EDTA; pH 8.3). Gels were run at 100 V for 4 h and stained with silver nitrate. An *MspI* digestion of pUC19 and M 100 bp were used as molecular weight marker (Fig. 2).

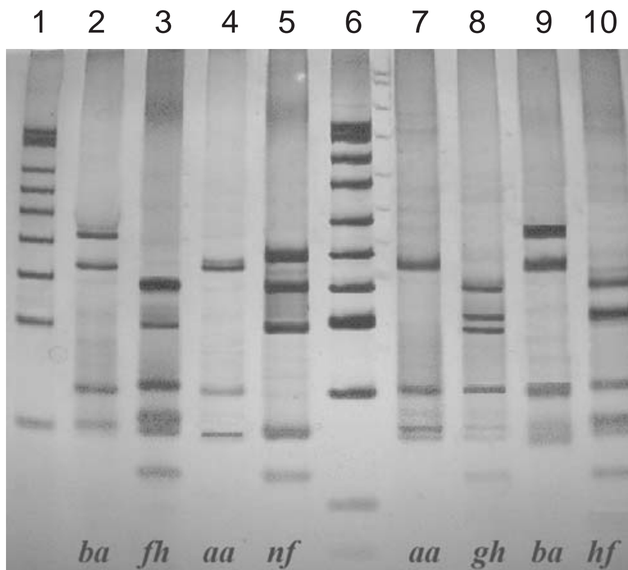

Figure 2. Restriction analysis of amplification products in exon2 of BoLA-DRB3 gene in 8% polyacrylamide gel. Lane 1: M50 molecular marker (500, 450, 400, 350, 300, 250, 200, 150, 100, 50 bp). Lanes 2, 4, 7, and 9 digested by *Hae*III. Lanes 3, 5, 8 and 10 digested by *Rsa*I. Lane 6: pUC19/*Msp*I molecular marker (501, 489, 404, 331, 242, 190, 147, 111, 110, 67, 34, 26 bp). fh (f: 141, 39, 54, 50 and h: 111, 69, 54,50 bp), aa (a: 167, 52, 65 and a: 167, 52, 65 bp), ba (b: 219, 65 and a:167, 52, 65 bp), nf (n:180, 104 and f: 141, 39, 54, 50 bp), hf (h: 111, 69, 54, 50 and f: 141, 39, 54, 50 bp) and gh (g:141, 39, 104 and h: 111, 69, 54, 50 bp).

***BoLA-DRB3* Typing:** *BoLA-DRB3.2* typing was performed using a PCR-RFLP method developed by Van Eijk *et al*. (1992). To date 93 alleles have been identified by restriction enzyme digestion of a 284 bp PCR product of DRB3 exon 2 and 103 alleles have been identified by PCR-sequence- based typing (SBT). The nomenclature for alleles of *BoLA-DRB3* defined by the PCR-RFLP method is indicated by the format locus.exon.allele, e.g., DRB3.2 *8 (Takeshima *et al*., 2001, 2003 and Da Mota *et al*., 2004).

RESULTS

We used a hemi-nested PCR-RFLP method for identification the frequency of *BoLA-DRB3*2* alleles in Iranian Sarabi cattle. PCR products were represented by 284 bp fragments as was expected on the basis of the nucleotide sequence of the gene (Fig.1). The spectra of *Rsa*I, *Hae*III and *Bsty*I restriction sites were shown by Van Eijk *et al*. (1992). Comparison of the restriction patterns obtained using the three endonucleases made it possible to identify 15 alleles of gene

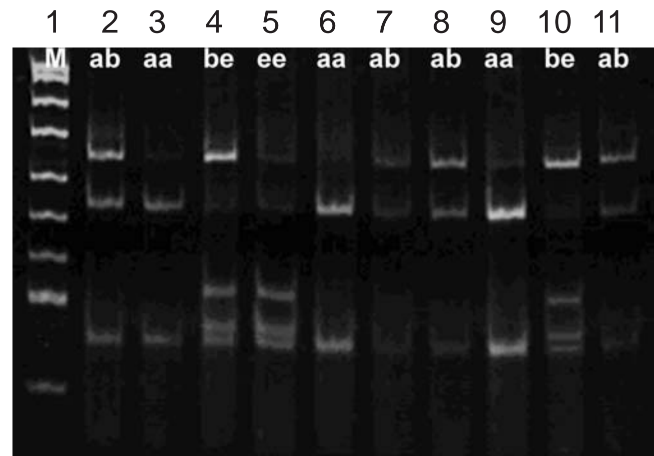

Figure 3. Restriction analysis of amplification products in exon 2 of BoLA-DRB3 gene in 8% polyacrylamide gel. Lane 1: pUC19/*Msp*I molecular marker. Lanes 2, 7, 8 and 11: ab (a: 167, 52, 65 and b: 284 bp). Lane 3, 6 and 9: aa (a: 167, 52, 65 bp). Lane 5: ee (e: 87, 112, 85 bp). Lanes 4 and 10: be (b: 284 and e: 87, 112, 85 bp).

DRB3 in our study (Table 1). The six most frequently isolated alleles (*BoLA-DRB3.2 *52, *11, *23, *12, *2,* and **43*) accounted for 78 % of the alleles in the population. The band patterns of the endonucleases *Rsa*I, *Hae*III are shown in figure 2, and *BstYI* are shown in figure 3. In the present study, *BoLA-DRB3.2*1, *4, *7, *10, *13, *15, *16* and other alleles were not detected and **52, *11, *23* and **12* were detected at a high frequency.

Table 1. Allele frequencies for bovine lymphocyte antigen DRB3.2 of 50 Sarabi cows as identified by polymerase chain reaction and restriction fragment length polymorphism analysis.

Patterns RsaI	*BstYI*	*HaeIII*	*DRB3.2* alleles (no.)*	*Frequency*
s	d	a	52	0.23
g	e	a	11	0.18
n	b	a	23	0.15
h	a	a	12	0.10
b	b	a	2	0.10
k	b	f	43	0.06
w	b	a	48	0.05
o	a	a	25	0.03
m	b	a	22	0.02
l	b	b	20	0.02
s	b	b	16	0.02
k	b	b	17	0.02
h	b	b	14	0.02
f	a	a	8	0.02
b	b	b	3	0.02

DISCUSSION

Results presented in this paper indicate that allelic frequencies of *BoLA-DRB3* to some extent, depend on the breed and population, as a result of the founder population and selection pressure. Therefore, more other *BoLA-DRB3* alleles can be identified in a large population. The method of DNA-typing of animals can be used in agricultural practice for *BoLA-DRB3* allele genotyping of cattle in order to reduce spreading of alleles providing susceptibility to mastitis or leukemia in cattle populations. Thus, investigation of DNA polymorphism for *BoLA-DRB3* gene may be essential for esstimation of practical, as well as theoretical value. This is the first report on the *BoLA- DRB3* gene in Iranian Sarabi cattle. Our present study demonstrated that the *BoLADRB3.2* locus is highly polymorphic in Sarabi cattle. A high degree of *BoLA-DRB3.2* polymorphism has also been reported in studies of Holstein, Jersey, Japanese Shorthorn, and Argentine Creole cattle (Takeshima *et al.*, 2002; Gilliespie *et al*., 1999; Dietz *et al*., 1997a, 1997b and Giovambattista *et al*., 1996). However, there are significant differences in frequencies of *BoLA-DRB3* alleles between Holstein, Jersey, Japanese Shorthorn, and Argentine Creole cattle. For example, the six most frequently detected alleles (*BoLA-DRB3.2 *8, *11, *16, *22, *23,* and **24*) accounted for 70.3 % of the alleles in a population of Holstein cows (Dieta *et al*., 1997b). Similarly, Sharif *et al.* (2003) showed that *BoLA-DRB3.2 *8, *11, *16, *22, *23, and *24* accounted for 83.5 % of the alleles in a population of Holstein cows in Canada. The six most frequently isolated alleles in Jersey cows were *BoLA-DRB3.2 *8, *10, *15, *21, *36,* and **ibe* and these accounted for about 74% of the alleles in the population. On the other hand, the most frequently detected BoLA-DRB3.2 alleles of 50 Sarabi cows were *BoLA-DRB3.2 *52, *11, *23, *12, *2* and **43*.

Sharif *et al*. (1998) found allelic frequencies of 20.1% for allele *DRB3.2*8* and 14.9% for *11 in Holsteins. Our study showed that frequency of *BoLA-DRB3.2**8 and *11 accounted for 2% and 18% respectivly, in Sarabi population. While in Jersi cattle allele *8 was present at a frequency of 11.3% and allele *11 was absent.

Alleles DRB3.2*48 and *43, which had a frequency of 5% and 6% respectiveley, in Sarabi cattle, were not reported in other breeds. Thus, it would appear that differences in allelic frequencies exist between the cattle breeds.

Significant association has been made with some infectious diseases of cattle and *BoLA* genes, particularly diseases that are prevalent during early lactation. For example, Schmutz *et al*. (1992) indicated that one *BoLA-DRB* gene pattern in a study of 106 Holstein cows was associated with resistance to *Staphylococcus aureus* mastitis. Associations between *BoLA* allele types and persistent lymphocytosis caused by bovine leucosis placenta, and *BoLA-DRB3.2*16* and *BoLA-DRB3.2*22* alleles were associated with a lower risk of cystic ovarian disease in Holstein dairy cows.

In the present study on Sarabi, *BoLA-DRB3.2*16* was not detected. However, Dietz *et al*. (1997b) reported that allele *16 was associated with increased risk of disease for infection. We also found that cows with alleles *DRB3.2*11, *12* or **23* which were more resistant to intramammary infections and the pathogenic effects of mastitic pathogens (Dietz *et al*., 1997a), Thus, Sarabi cattle shows a high degree of genetic potential for disease resistance.

Results of the present study indicate that differences exist between breeds of cattle with regard to *BoLADRB3.2* allelic frequency. Further analysis needs to be conducted on allelic patterns reported in this study to determine their nucleotide sequence. Studies are in progress to evaluate the relationship of *BoLA-DRB3.2* allele types in this Sarabi herd with Somatic Cell Count, mastitis susceptibility or resistance, and reproductive performance.

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